

Survival of Navel Orangeworm (Lepidoptera: Pyralidae) During Pistachio Processing

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ABSTRACT The potential survival of the navel orangeworm, *Amyelois transitella* (Walker), in pistachios after commercial processing was estimated by sampling nuts from 2 different driers. In total, 1,980 kg of pistachios (≈880,000 open nuts) were sampled over 3 yr. No live navel orangeworm were found and only 1 dead adult was discovered. Samples from drum driers, which received floaters, had an average of 10.7 dead navel orangeworm per kilogram of nuts, whereas samples from the fan driers had an average of 1.9 dead navel orangeworm per kilogram of nuts. The occurrence of moths in traps baited with virgin, laboratory-reared females suggests that some navel orangeworm survive processing, but it was not possible to determine the source of the moths. Commercial pistachio drying was simulated in a forced-air oven with an air temperature of 90°C. Nut temperatures during laboratory oven drying exceeded 60°C after 45 min, when nut moisture levels were >26%. Nut temperatures were >60°C for 2.25 h before nut moisture content approached appropriate storage levels. Limited survival of navel orangeworm eggs (2.82%) and pupae (3.33%) occurred after 30 min of oven exposure, when not all nut temperatures had exceeded 60°C. No navel orangeworm survived oven exposures of 1 h or longer. Our results show that survival of navel orangeworm in processed pistachios is very low and should not be of major concern to processors.

KEY WORDS *Amyelois transitella*, pistachio, postharvest, high-temperature

THE UNITED STATES produces >30% of the world's pistachios, and is 2nd only to Iran in total production. All domestic pistachio production occurs in California, where 67.5 million kilograms (150 million pounds), with a value of nearly \$450 million, was produced in 1993 (California Pistachio Commission 1994). Because ≈75% of California pistachios are marketed as an in-shell, out-of-hand snack food (Crane and Maranto 1988), quality is dependent on careful production and processing methods that produce large nuts with opened, unblemished shells. High quality pistachios justify a higher price on the export market.

Timely harvesting, hulling, and drying of pistachios is necessary to prevent staining of the shells and to maintain high quality. The practice of using red dye on pistachio shells was begun to cover unsightly stains resulting from poor processing. The improved methods used by California pistachio processors has all but eliminated the need to dye the product. Pistachio processing begins with removal of the hull from the shell. After hulling, nuts are placed in water tanks to float off the lighter blank, closed, or damaged nuts from higher quality nuts. Immediately after flotation, both fractions of

the nuts are dried, reducing the moisture content from ≈40% to ≈5%. Afterwards, the lighter floaters, which are closed, blank, or severely damaged, are normally shelled and used in confectionary products. The most common drying technique is to heat the nuts with air temperatures of 66–82°C for 8–10 h in large rotary drum driers. A newer, energy-efficient, 2-stage method uses modified grain driers (air temperatures from 70 to 90°C) to dry the nuts to 9–12% moisture in < 2 h, then completes the drying process with forced warm air while the nuts are in storage silos. Dried pistachios may be stored at ambient temperatures for several months before final sorting, roasting, and marketing.

The navel orangeworm, *Amyelois transitella* (Walker), is a common pest of tree nuts in California and is the major insect pest of pistachios (Rice 1978). Navel orangeworm is primarily a field pest and low level infestations may be present at harvest. Casual observations of nuts sampled just after flotation have shown that many navel orangeworm are alive in the pistachios as they enter the driers. Although navel orangeworm should not survive the high temperatures used for drying, processors often relate anecdotal evidence to the contrary. Closer study has shown that because of evaporative cooling nutmeat temperatures may not approach air temperatures until the nuts are nearly

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dried. Variation in drying efficiency throughout the product mass may result in some nuts experiencing lower temperatures. Thus, some navel orangeworm survival is possible, particularly in the 2-stage drying method, which does not completely dry the nuts in the initial high-temperature drying stage.

Fumigation of stored pistachios with methyl bromide is the usual method to control infestations of postharvest insects. Processors normally fumigate storage silos shortly after drying, primarily to control infestations of Indianmeal moth, *Plodia interpunctella* (Hübner). Some processors are also concerned with additional damage caused by surviving navel orangeworm larvae and the possibility of continued navel orangeworm reproduction in the storage environment (Hartsell et al. 1986). Methyl bromide was recently classified as an ozone depleter (EPA 1993), which may result in its use being severely restricted or eliminated. Insect resistance to phosphine, the only available alternative fumigant, has been documented in other commodities (Zettler et al. 1989). Some of the proposed alternative control methods are specific to Indianmeal moth (Sower et al. 1975, Hunter et al. 1977, Vail et al. 1991) and would not be effective against navel orangeworm. In the current study we show that survival of navel orangeworm in processed pistachios is very low, and should not be of major concern to processors.

Materials and Methods

The study was done at a large pistachio plant near Madera, CA. The plant typically handles between 4.1 and 5.4 million kilograms of pistachios each year. We sampled nuts during normal processing in September of 1991, 1992, and 1993. During the study, the processor used the 2-stage method to dry *sinkers*, which are the higher quality nuts that sink in the flotation tanks. Continuous flow, 3-level fan driers were used for the initial drying stage. Average air temperatures were 71, 77, and 88°C for the bottom, middle, and top levels, respectively. Nuts took from 45 to 90 min to pass through the drier. Optimal moisture contents for nuts exiting the fan driers were between 9 and 12%, although measurements ranged from 6 to 14%. Nuts that did not sink in the flotation tanks, *floaters*, were dried in batch drum driers with air temperatures of 82°C for ≈8 h. Optimal moisture content for these nuts was 5% but would range as high as 7–8%.

Sampling Procedures. We began preliminary sampling in 1991. Twenty 4.5-kg samples were taken on 26 and 27 September, from the output of both the fan and drum driers. Each sample was placed in plastic boxes (38.1 by 25.4 cm) covered with fine cotton organandy cloth. The nuts were held at 28°C and ambient relative humidity and checked periodically for adult navel orangeworm emergence. After 4 wk, numbers of nuts in each

sample were estimated. Nuts that appeared damaged or infested were opened and examined for the presence of navel orangeworm larvae and pupae. Comparisons between the driers were made with SAS PROC TTEST (SAS Institute 1987) for numbers of larvae, pupae, total navel orangeworm, and damaged nuts. Paired *t*-test comparisons of larvae and pupae were made within each drier with SAS PROC MEANS (SAS Institute 1987).

More extensive sampling was done in 1992 and 1993. Five samples were taken from both the fan and the drum driers each year. With 1 exception, all samples from the driers were taken on different days. Each sample consisted of four 22.5-kg subsamples placed in 0.128-m³ plastic garbage cans. Immediately after collection, each subsample was shaken over a modified raisin shaker, and the resulting debris examined for dead or living insects. At this time, 2.25-kg samples were taken from each subsample. Open and closed nuts in the small samples were counted, and all open nuts shelled and examined for insect infestation and damage.

The remainder of the 22.5-kg samples were held in modified plastic garbage cans under a shade cover at ambient temperatures. Ventilation was improved by cutting 3 holes (5 cm diameter) ≈5 cm below the rim of each can and covering the holes with 150-mesh stainless steel screen. Cans used for samples from the fan driers had false bottoms made of galvanized wire fencing supported 9 cm above the bottom of the cans by pine blocks. A 5-cm hole was cut into the side of each can below the false bottom, and a length of flexible pipe fitted into the hole. The other end of the pipe was attached to a modified evaporative-cooler fan, which forced air through the samples for 48–72 h to reduce their moisture content and prevent mold development. Nuts sampled from the drum driers did not need additional ventilation. Each can was inspected periodically for adult navel orangeworm. After ≈4 mo, subsamples were again shaken on the raisin shaker, and the debris was examined for insects.

For analysis, data from the four 2.25-kg subsamples of each sample were combined. The proportion of open nuts in each 9.0-kg sample was used to derive a correction term which standardized the samples at 10 kg of open nuts. The correction term was used to adjust the number of larvae, pupae, total insects, and damaged nuts. Because variances for the driers were found not to be consistent, analysis was performed on log transformed data. SAS procedure analysis of variance (ANOVA) (SAS Institute 1987) was used to compute a 2-way ANOVA for randomized complete block design with years as a blocking factor. Paired *t*-test comparisons of larvae and pupae were made within each drier with SAS PROC MEANS (SAS Institute 1987).

Pheromone Trapping. Navel orangeworm populations were monitored at the processing plant with Pherocon 1C sticky traps (Zoëcon, Palo Alto,

CA) baited with virgin female moths from a laboratory culture. Five female pupae were placed in cages made by heat-sealing the edges of nylon window screen to form pyramidal bags (4 by 4 cm) (Curtis and Clark 1984). The cages were hung on wire clips placed under the tops of the traps. Traps were monitored each week; we selected pupae of various ages to ensure a continuous supply of calling females throughout the week. In 1991, six traps were placed near processing areas, and in and around storage silos. The traps were first set out 19 September, just before processing began, and were maintained until 24 November. In 1992, eight traps were set up on 27 August, ≈ 10 d before processing began, and maintained until 15 October. In 1993, six traps were set out on 12 August, ≈ 4 wk before processing began, and were maintained until 2 December.

Laboratory Drying of Infested Pistachios. We collected hulled pistachios from flotation tanks at the commercial processor and infested them with navel orangeworm eggs and pupae from our laboratory culture. The test was done once each week for 3 wk, using freshly collected nuts each time. Single paper toweling strips with 25 eggs (42 ± 6 h old) were placed between the shell and meat of each nut. Other nuts were infested with single pupae placed in holes drilled into the meat with a 3-mm bit. Infested nuts were placed in 5 baskets (25.5 by 5 by 10.5 cm) made from 6.5-mm hardware cloth. Ten nuts infested with eggs, 25 nuts infested with male pupae, and 25 nuts infested with female pupae were put in each basket. Uninfested nuts were added to fill each basket, with infested nuts scattered evenly throughout. Ten egg strips, 25 male and 25 female pupae were used as untreated controls. Control eggs were placed in a plastic petri dish (9.0 cm diameter) filled with a wheat bran diet modified from that described by Finney and Brinkman (1967) (Tebbetts et al. 1978). Control pupae were placed in similar dishes lined with filter paper.

Four baskets were placed lengthwise 3 cm in front of the air input of a forced air oven with an air temperature of 90°C. One basket each was removed after 0.5, 1, 2, and 3 h of oven exposure. Immediately after removal from the oven, the nuts were placed in enamel pans (25 by 40 by 6 cm). Nuts in the remaining basket (0 h) were kept at ambient temperatures ($\approx 25^\circ\text{C}$) for 3 h before placing them in an enamel pan. After all the baskets were removed from the oven, eggs and pupae were removed from infested nuts and placed in dishes similar to those used for untreated controls. All dishes were held at 25°C, 60% RH, and a photoperiod of 14:10 (L:D) h and examined regularly for egg hatch or adult emergence.

Immediately after removal of each basket from the oven, ≈ 25 nuts were shelled and chopped and their moisture content determined with an infrared moisture determination balance (Ohause, Florham Park, NJ). Moisture content for nuts from the 0-h

Table 1. Recovery of navel orangeworm from 4.5-kg samples of pistachios taken from 2 commercial driers in 1991

| Stage recovered | No. insects or nuts per 4.5-kg sample | |
|------------------------|---------------------------------------|--------------------|
| | Fan drier ^a | Drum drier |
| Live navel orangeworm | 0 | 0 |
| Dead larvae | $7.95 \pm 0.94^*$ | $26.05 \pm 3.06^*$ |
| Dead pupae | $2.05 \pm 0.34^*$ | $22.30 \pm 2.47^*$ |
| Total navel orangeworm | $10.05 \pm 1.05^*$ | $48.35 \pm 5.23^*$ |
| Total damaged nuts | $13.5 \pm 1.34^*$ | $22.4 \pm 1.89^*$ |

* Significant difference between means of driers ($P > 0.05$ level, *t*-test [SAS Institute 1987]).

^a A single dead moth was found in 1 fan drier sample.

basket was determined when the other baskets were placed in the oven.

Nutmeat and shell temperatures were recorded during drying with thermocouples made from 36-gauge, copper-constantan thermocouple wire. Thermocouples were attached to each of 8 nuts by threading the wire through 3 holes drilled in the shell. Four nuts, 2 with thermocouples placed between the nutmeat and shell and 2 with thermocouples placed in the center of the nutmeat, were wired to the inside of the front of the last basket to be removed from the oven. Four similar nuts were wired to the inside of the back of the same basket. Another thermocouple was placed inside the oven to measure air temperature. Temperatures were recorded every 20 s with a Polycorder datalogger (OmniData, Logan, UT).

Results

Nut Samples. The pistachio samples taken in 1991 from the fan and drum driers contained $\approx 3,000$ and 3,300 nuts per 4.5 kg, respectively. More nuts were found in the drum samples because there was a higher proportion of blank nuts, and because their lower moisture content caused individual nuts to weigh less. No living navel orangeworm of any stage was found in any of the samples (Table 1). A single dead navel orangeworm adult was found in a fan drier sample. The adult was found in the bottom of the sample container, but no pupal exuvia was recovered from any of the nuts in that sample. It was suspected that the adult may have entered the sample after processing.

Significantly more dead navel orangeworm were found in drum drier samples than in fan drier samples ($t = -8.14$ to -3.84 ; $df = 20, 34$; $P < 0.001$). In the drum drier, the number of navel orangeworm found was greater than the number of damaged nuts, indicating that 2 or more navel orangeworm were often found in a single damaged nut. Samples from the fan drier had significantly more larvae than pupae ($t = 6.35$, $df = 39$, $P = 0.0001$). There was no significant difference between number of larvae and pupae found in samples from the drum drier ($t = 1.99$, $df = 39$, $P = 0.061$).

Table 2. Recovery of navel orangeworm from pistachio samples taken from 2 commercial driers in 1992 and 1993

| | Fan drier | | Drum drier | |
|----------------------------|---------------------|------------------|---------------------|---------------------|
| | 9-kg sample | 10 kg open nuts | 9-kg sample | 10 kg open nuts |
| Total nuts | 6,477.1 \pm 125.0 | — | 9,693.4 \pm 560.3 | — |
| Open nuts | 6,079.3 \pm 181.1 | — | 1,923.9 \pm 336.6 | — |
| Damaged nuts | 35.6 \pm 4.45 | 42.4 \pm 5.82* | 109.7 \pm 27.42 | 573.4 \pm 105.26* |
| Dead navel orangeworm | | | | |
| Pupae | 4.2 \pm 1.51 | 4.9 \pm 1.71* | 59.4 \pm 16.92 | 354.3 \pm 99.10* |
| Larvae | 10.7 \pm 1.15 | 12.6 \pm 1.40* | 37.2 \pm 10.34 | 211.5 \pm 85.72* |
| Total | 14.9 \pm 2.11 | 17.5 \pm 2.43* | 96.6 \pm 26.99 | 565.8 \pm 181.20* |
| Live navel orangeworm | 0 | 0 | 0 | 0 |
| Navel orangeworm in debris | | | | |
| Dead | | 2.1 \pm 0.64 | | 4.7 \pm 1.14 |
| Alive | | 0 | | 0 |

Values are means \pm SEM. Values for debris were obtained after 90-kg samples were passed over a modified raisin shaker. *, Significant difference between means of driers for 10 kg open nut estimates ($P > 0.01$ level, ANOVA [SAS Institute 1987]).

Results of samples taken in 1992 and 1993 are summarized in Table 2. Samples from the fan drier had a much higher proportion of open nuts ($93.7 \pm 1.14\%$) than samples from the drum drier ($21.8 \pm 4.33\%$). Because only open nuts were considered to be susceptible to infestation, comparisons between the 2 driers were made based on a standard weight (10 kg) of open nuts. Significantly more pupae ($F = 171.2$, $df = 1$, $P = 0.0001$), larvae ($F = 10.4$, $df = 1$, $P = 0.005$), total navel orangeworm ($F = 150.4$, $df = 1$, $P = 0.0001$), and

damaged nuts ($F = 156.0$, $df = 1$, $P = 0.0001$) were found in samples from the drum drier. No live navel orangeworm of any stage nor dead adults were found in any sample from either drier.

As in the earlier study, samples from the fan drier had significantly more larvae than pupae ($t = 3.92$, $df = 19$, $P = 0.003$). In contrast, samples from the drum drier had significantly more pupae than larvae ($t = 3.68$, $df = 39$, $P = 0.005$), because the drum drier received floaters. Pistachios containing navel orangeworm pupae were more often severely damaged than those with larvae, and are thus more likely to be dried in the drum drier. Flotation serves to remove pistachios infested with pupae from nuts dried in the fan drier.

No live moths were detected in any of the 90-kg samples during the 4 mo they were held after sampling. Only dead navel orangeworm were recovered from debris shaken from the samples. Average number of insects found in the debris was very low (Table 2). There was no significant difference between navel orangeworm recovered from the 2 driers ($F = 3.87$, $df = 1$, $P = 0.067$). Most important is the fact that no dead adults were recovered, even after 4 mo post sample, indicating that no immature navel orangeworm in any of the 90-kg samples survived processing.

Pheromone Trapping. Relatively low numbers of male navel orangeworm were caught in traps baited with laboratory-reared virgin females (Fig. 1). A total of 7 moths was caught in 1991, and moths were only found in traps near incoming nuts, processing areas, or storage silos. No moths were caught after processing ended. In 1992, 2 moths were caught during the 3rd wk of monitoring, ≈ 2 wk after processing began. No other moths were caught that year. More navel orangeworm were caught in 1993; a total of 27 moths was caught over a 16-wk period. One moth was recovered before product was brought into the plant, indicating that moths may fly into the processing area from surrounding fields. Eleven moths were caught in the 4 wk after processing ended, and

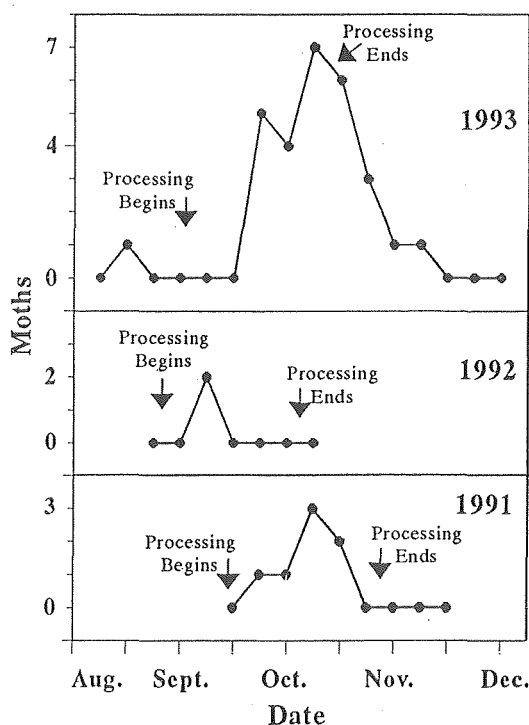


Fig. 1. Numbers of male navel orangeworm caught in virgin female baited traps during pistachio processing, 1991-1993.

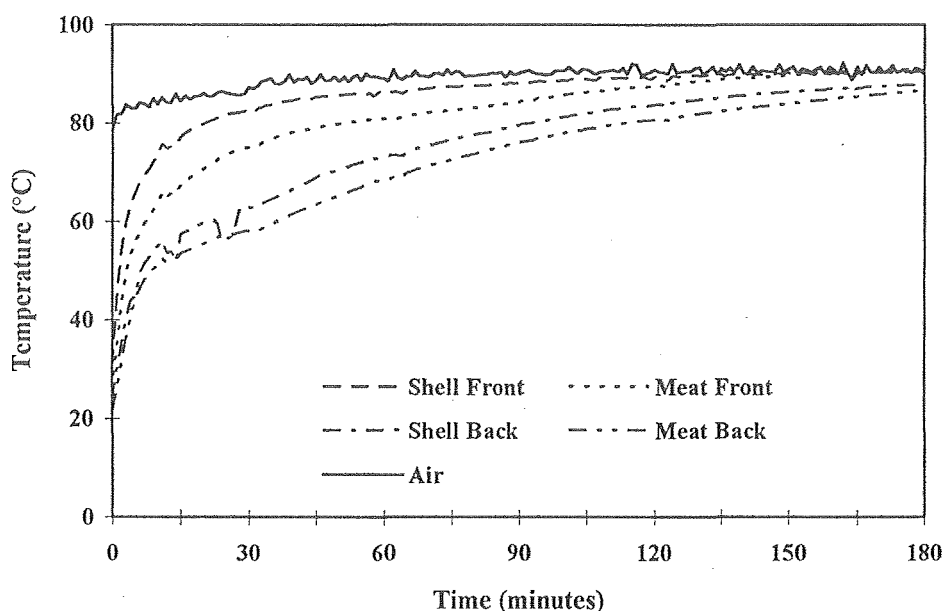


Fig. 2. Pistachio nut temperatures during laboratory drying in a forced-air oven with an air temperature of 90°C.

may have emerged from silos before product was fumigated.

Laboratory Drying of Infested Pistachios. Temperature increase of nuts during oven drying varied with their position in the baskets (Fig. 2). Nut temperatures in the front of the baskets, directly in front of the air input, increased at a faster rate than nut temperatures in the back of the baskets. Temperatures between the shell and meat increased faster than temperatures in the center of the meat. All recorded temperatures exceeded 60°C after 45 min of oven exposure. After 3 h of oven exposure, nut moisture content decreased from $\approx 42\%$ to 7.6% (Table 3). The final moisture level is comparable to that obtained in the fan drier. No navel orangeworm eggs or pupae survived 1 h or more of oven exposure. After 30 min of oven exposure, survival of eggs and pupae was 2.82 and 3.33%, respectively.

Discussion

Temperatures $>50^{\circ}\text{C}$ are considered to be almost immediately lethal to most insects (Bursell 1974). Curtis et al. (1984) noted that a high pro-

portion of navel orangeworm larvae, pupae, and eggs were killed in almonds exposed to direct sunlight, where maximum temperatures of 57.8°C were recorded. The estimated LT_{95} for both navel orangeworm eggs and pupae at 49°C was 11 min, and survival of larvae and pupae in heat-treated, in-shell walnuts was 32% after 10 min at 50°C (J.A.J., unpublished data). During drying, pistachios are exposed to air temperatures of from 66 to 90°C for 1–8 h, depending on the type of method used. Kader et al. (1980) showed that as pistachios reach storage moisture levels (3–8%) final nut temperatures were very near the drying temperature. In our study, nut temperatures during laboratory drying exceeded 60°C after 45 min, when nut moisture levels were $>26\%$. Nut temperatures were $>60^{\circ}\text{C}$ for 2.25 h before nut moisture content approached appropriate storage levels. It seems unlikely that any navel orangeworm could survive normal drying procedures, and the results from our laboratory drying studies support this conclusion. Limited survival of navel orangeworm occurred only after 30 min of oven exposure, when not all nut temperatures had exceeded 60°C . The occurrence of moths in pheromone traps suggests

Table 3. Survival of navel orangeworm eggs and pupae in laboratory-dried pistachios ($n = 3$)

| Treatment, h | % Egg hatch (mean \pm SEM) | % Adult emergence (mean \pm SEM) | Nut temp, $^{\circ}\text{C}$ | % Moisture content (Mean \pm SEM) |
|--------------|---------------------------------|---------------------------------------|------------------------------|--|
| Control | 93.7 \pm 0.26 | 96.7 \pm 0.37 | — | — |
| 0 | 86.1 \pm 0.64 | 82.7 \pm 1.84 | 21.3–30.4 | 42.2 \pm 0.07 |
| 0.5 | 2.82 \pm 1.55 | 3.33 \pm 0.97 | 58.1–82.5 | 35.9 \pm 0.32 |
| 1.0 | 0 | 0 | 68.4–86.0 | 26.5 \pm 1.03 |
| 2.0 | 0 | 0 | 80.8–89.4 | 13.4 \pm 0.45 |
| 3.0 | 0 | 0 | 86.1–89.4 | 7.6 \pm 0.33 |

that some navel orangeworm may survive processing, but it was not possible to determine the source of the moths. Moths may have emerged from nuts that were not yet processed, from spilled nuts, from empty bins, or from sites outside the plant.

Our study shows that most navel orangeworm-infested nuts are concentrated in the floated fraction during processing. Samples from drum driers, which received floaters, had an average of 10.7 navel orangeworm per kilogram of nuts, whereas samples from the fan driers had an average of 1.9 navel orangeworm per kilogram of nuts. Floatation removes roughly 30% of incoming nuts, resulting in $\approx 71\%$ of the navel orangeworm being concentrated in $<1/3$ of the nuts. In the 3 yr of the study, we sampled 1,980 kg of pistachios, or an estimated 880,000 open nuts. Within these nuts, no live navel orangeworms were found and only 1 adult moth was discovered in nuts from the fan drier. Because the fan drier does not dry the product to the final storage moisture of 5%, it may be that the chance of navel orangeworm survival is greater, even though a higher air temperature is used. However, incidence of survival in our samples was too low to make this determination. Therefore, we estimated a possible survival rate of 1 navel orangeworm per 2,000 kg of nuts, regardless of drying method. Studies on navel orangeworm damage in almonds showed that older larvae cause no further losses after harvest, because the larvae have already transformed the nutmeats to rejects and do not seem to move between nuts (Curtis et al. 1984). Similarly, little direct damage to postharvest pistachios should result from continued feeding by surviving larvae.

Of greater concern to processors is the possibility that emerging moths might reinfest the product. Using the above survival estimate, between 2,000 and 3,000 moths would be expected to emerge each year, or ≈ 400 –750 wk, at the processing plant in the study. This emergence would occur throughout the storage silos. Recent studies with codling moth, *Cydia pomonella* (L.), showed that mating success of single pairs of moths in 22.5-kg bags of in-shell walnuts was very low, usually $<7.1\%$ for newly isolated cultures (Curtis et al. 1992). Moths apparently had difficulty in finding one another within the nuts. Mating success of navel orangeworm emerging deep within the nuts in pistachio silos, at an estimated density of 1 pair per 4,000 kg of nuts, should also be very low. Successful mating would probably be limited to those moths emerging near the product surface and able to escape into the silo headspace.

The adverse effect of heat applied to developmental stages of several species of Lepidoptera on their subsequent reproduction has been documented (Proverbs and Newton 1962, Sugai and Ashoush 1968, Guerra 1972). Lum (1977) found that eupyrene sperm development was inhibited in Indianmeal moth pupae and prepupae exposed to 35°C for >3 d, resulting in a reduction in egg pro-

duction. Fecundity of Indianmeal moth and *Cadra cautella* (Walker) was reduced in adults exposed as pupae to 40, 45, or 50°C (Arbogast 1981). Progeny numbers were reduced by $>70\%$ when either male or female Indianmeal moth were exposed as young pupae to 46°C for 10 min (Johnson et al. 1992). Therefore, a portion of the navel orangeworm that survive the high temperatures found in pistachio driers may be sterile. Moreover, there is little evidence that navel orangeworm are capable of reinfesting nuts in storage. Michelbacher and Davis (1961) implied that navel orangeworm can reproduce in stored walnuts under favorable conditions, but Post et al. (1959) demonstrated that reproduction of navel orangeworm in stored almonds is unlikely.

At the time of pistachio processing, high numbers of Indianmeal moth are often caught in nearby pheromone traps (J.A.J., unpublished data). These moths are well adapted to storage conditions and present an immediate threat to the nuts. Most processors direct their postharvest fumigation at this pest. It is possible, however, that the belief by a few processors that navel orangeworm survives processing and reproduces in stored pistachios is a result of an inability to differentiate between the 2 moths. The pending loss of general biocidal fumigants has generated a need to better understand the dynamics of the postharvest environment. Our study shows that navel orangeworm need not be considered a potential pest after processing.

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